NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Total RNA Purification Plus Micro Kit — Product Insert

Product # 48500

Norgen's Total RNA Purification Plus Micro Kit provides a rapid and sensitive method for the isolation and purification of total RNA from small input amounts of cultured animal cells, tissue samples, and microdissected samples including laser-capture microdissection (LCM). The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). Genomic DNA is removed from the sample using a Genomic DNA Removal column, and the RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the cells or tissue of interest with the provided Buffer RL (please see the flow chart on page 4). The DNA is then captured on a Genomic DNA Removal Column. Ethanol is then added to the flowthrough of the DNA removal step, and the solution is loaded onto an RNA Purification Micro Column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution A. The special design of the micro spin-column allows a small elution volume of as little as 20 µL. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	35 µg
Maximum Column Loading Volume	650 µL
Minimum Elution Volume	20 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Animal Tissues Laser-Captured Microdissection (LCM)	 5×10^5 cells 3 mg (for most tissues*) Up to 5×10^5 cells
Time to Complete 10 Purifications	22 minutes
Average Yields HeLa Cells (1×10^5 cells)	 1.5 µg

***** *for fibrous tissue, an additional Proteinase K treatment is required*

Advantages

- Fast and easy processing using rapid micro spin-column format
- Small elution volume of 20 µL
- Isolate total RNA, from large rRNA down to microRNA (miRNA) without compromising total yield
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of sources
- Rapidly remove contaminating genomic DNA without the use of enzymes
- RNA can be isolated and detected from as little as a single animal cell

Kit Components

Component	Product # 48500 (50 preps)
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Genomic DNA Removal Column	50
RNA Purification Micro Column	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Buffer RL** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Total RNA Purification Plus Micro Kit:

For All Protocols

- Benchtop microcentrifuge
- 96 - 100% ethanol
- β-mercaptoethanol (optional)

For Animal Cell Protocol

- PBS (RNase-free)

For Animal Tissue Protocol

- Liquid nitrogen
- Mortar and pestle
- 70% ethanol

For Laser-Captured Microdissection (LCM) Protocol

- Sterile fine forceps
- Water bath or heat block set at 42^{o}C

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Flowchart

Procedure for Purifying Total RNA using Norgen's Total RNA Purification Plus Micro Kit

A. Genomic DNA Removal

Lyse cells or tissue using **Buffer RL**

Eliminate DNA using the Genomic DNA Removal Column

SPIN

Flowthrough (with RNA)

Add ethanol

B. Purification of RNA

Bind to RNA Purification Micro Column

SPIN

Wash three times with Wash Solution A

SPIN

Elute RNA with Elution Solution A

SPIN

Purified Total RNA

Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Section 1. Preparation of Lysate From Various Cell Types

Notes Prior to Use

- The steps for preparing the lysate are different depending on the starting material **(Step 1)**. However, the subsequent steps are the same in all cases **(Steps 2 – 6)**.
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Optional:** The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (ex: pancreas), including LCM samples. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Buffer RL required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the Buffer RL can be used as provided.
- It is important to work quickly during this procedure.

1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- The maximum recommended input of cells is 5×10^5. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, each well of a confluent 12-well plate of HeLa cells will contain 5×10^5 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Buffer RL directly to the frozen cell pellet **(Step 1A(ii) c).**

1A(i). Cell Lysate Preparation from Cells Growing in a Monolayer

a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
b. Add 350 µL of **Buffer RL** directly to culture plate.
c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
d. Transfer lysate to a microcentrifuge tube.
e. **Proceed to Step 2.**

1A (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 *x g* (~2,000 RPM) for 10 minutes to pellet cells.
b. Carefully decant the supernatant.

> **Note:** For inputs of over 10^5 cells, 5-10 µL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged. For inputs of fewer than 10^5 cells, 30-50 µL of media may be left behind in order to ensure that the pellet, which could be invisible, is not dislodged.

c. Add 350 µL of **Buffer RL** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
d. **Proceed to Step 2.**

1B. Lysate Preparation from Animal Tissues

Notes Prior to Use

- Norgen's Total RNA Purification Plus Micro Kit is designed for isolating RNA from small amounts of non-fibrous tissue samples (up to 3 mg in most cases). If a larger amount of starting material or fibrous tissue is desired, an additional Proteinase K treatment is required. Please refer to Appendix A for instruction.
- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.
- Tissues stored in RNA stabilization reagents such as RNA*later*® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry excessive liquid.
- The maximum recommended input of tissue varies depending on the type of tissue being used. Please refer to Table 1 below as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below we recommend starting with an input of no more than 3 mg.

Table 1. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Brain, Kidney, Liver, Lung, Spleen	3 mg
Heart, Muscle	Refer to Appendix A

1B. Cell Lysate Preparation from Animal Tissues

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing. Please refer to Table 1 for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 3 mg.
c. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e. Add 400 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.
f. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
g. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
h. **Proceed to Step 2.**

1C. Lysate Preparation from Laser-Captured Microdissection (LCM)

Notes Prior to Use

- LCM samples obtained from frozen sections are recommended. Formalin-Fixed, Paraffin-Embedded sections may also be used. However, RNA isolated from FFPE samples generally has poorer quality than that from frozen sections.

1C. Cell Lysate Preparation from Laser-Captured Microdissection (LCM)

a. Aliquot 300 µL of **Buffer RL** to an RNase-free microcentrifuge tube.
b. Remove the thermoplastic film containing the captured cells using sterile fine forceps. Carefully submerge the sample into the aliquoted **Buffer RL**. Close the microcentrifuge cap.
c. Incubate the sample at 42^{o}C for 30 minutes. Apply vortex for 15 seconds after every 10 minutes.
d. At the end of the incubation, vortex the tube one more time for 15 seconds. The thermoplastic film may be removed at this point using sterile fine forceps.
e. **Proceed to Step 2.**

Section 2. Genomic DNA Removal from All Types of Lysate

Notes

- The remaining steps of the procedure for the purification of total RNA are the same from this point forward for all the different types of lysate.
- This kit is provided with 2 separate columns. When columns are removed from the labeled bags they are supplied in they can easily be identified as follows:
    - **gDNA Removal Columns** - provided with collection tubes attached, column has predominately white contents
    - **RNA Purification Micro Columns** – collection tubes provided separately, column has predominately black contents

2. Genomic DNA Removal

a. Retrieve a gDNA Removal Column pre-assembled with a collection tube.
b. Apply up to 600 µL of the lysate prepared from Section 1 onto the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

c. **Retain the flowthrough for RNA Purification (Section 3)**. The flowthough contains the RNA and should be stored on ice or at -20°C until the RNA Purification protocol is carried out.
d. Dispose of the gDNA Removal Column with the bound gDNA.

Section 3. Total RNA Purification from All Types of Lysate

3. Binding RNA to Column

a. To every 100 µL of flowthrough from Step **2c**, add 60 µL of 96 – 100 % Ethanol. Mix by vortexing.

Note: For example, for 300 µL of flowthrough, add 180 µL of 96 – 100 % Ethanol

b. Assemble an RNA Purification Micro Column with one of the provided collection tubes.
c. Apply up to 600 µL of the lysate with the ethanol onto the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

d. Depending on your lysate volume, repeat Step **3b** and **3c** as necessary.

4. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **4a** and **4b** to wash column a second time.
d. Repeat steps **4a** and **4b** to wash column a third time.
e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 40 µL of **Elution Solution A** to the column.

Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 µL is recommended

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b and 5c**).

6. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Appendix A

Lysate Preparation from Animal Tissues with the use of Proteinase K

Customer-Supplied Reagent

- RNase-Free Proteinase K
- RNase-Free Water
- β-mercaptoethanol

Notes Prior to Use

- Ensure that all solutions are at room temperature prior to use.
- We recommend the use of Norgen's **Proteinase K** (Cat # 17904) for this step. Reconstitute each of the **Proteinase K** vials in 600 µL of molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free). For every isolation, 20 µL of the reconstituted Proteinase K is needed. Aliquot the remainder into small fractions and store the unused portions at -20°C until needed.
- If using another source of **Proteinase K,** reconstitute in molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free) to give a 20 mg/mL final concentration. For every isolation, 20 µL of the reconstituted Proteinase K is needed. Aliquot the remainder into small fractions and store the unused portions at -20°C until needed.
- Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Buffer RL** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.
- Tissues stored in RNA stabilization reagents such as RNA*later*® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry any excessive liquid.
- This protocol is particularly suitable for isolating RNA from up to 7.5 mg of tissues including fibrous, connective tissues.

Cell Lysate Preparation

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing.
c. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.

   Note: The use of liquid nitrogen is recommended. However, if homogenization without flash-freezing is preferred, proceed to Step **e**.

d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

e. Add 300 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.

   Note: Maximum homogenization may be achieved by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.

f. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
g. Add 600 µL of **RNase-Free Water** (not provided) to the lysate. Vortex to mix.
h. Add 20 µL of reconstituted Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation.
i. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume.
j. **Proceed to Step 2.**

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Buffer RL was used for the amount of cells or tissue.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the RNA Purification Micro column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100 % ethanol is added to the supplied Wash Solution A prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure that the cell monolayer is washed with the appropriate amount of PBS in order to remove residual media from cells.
	LCM: Sample was not incubated at 42^{o}C for 30 minutes	Ensure that the incubation at 42^{o}C for the removal and lysis of cells from the thermoplastic film.

Problem	Possible Cause	Solution and Explanation
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue. Perform the optional Proteinase K treatment for fibrous tissues
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the Genomic DNA Removal column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Buffer RL.

Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
Proteinase K – 2 Vials	17904
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Total RNA Purification 96-Well Kit	24300
Total RNA Purification Maxi Kit	26800
Animal Tissue RNA Purification Kit	25700
Plant/Fungi Total RNA Purification Kit	25800
RNA/Protein Purification Kit	24100
RNA/DNA/Protein Purification Kit	24000
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

©2014 Norgen Biotek Corp.